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Assessment of toxicities of sixteen insecticides to the plant bug, *Apolygus lucorum* (Hemiptera: Miridae) by glass-vial and artificial diet bioassays

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Abstract:【Aim】*Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae) is a serious piercing sucking pest of Bt cotton in China. Currently, spraying organophosphates and pyrethroids has been widely accepted for *A. lucorum* management, while spraying neonicotinoids is not recommended due to their low contact toxicity. However, neonicotinoid insecticidal seed treatments effectively protect cotton from *A. lucorum* infestation until blooming stage. Therefore, an appropriate bioassay method needs to be developed to re-evaluate the toxicity of these insecticides to *A. lucorum*.【Methods】Two different types of bioassays, *i. e.*, glass-vial and artificial diet toxicity bioassay, were used to assess the toxicities of 16 insecticides belonging to five chemical categories to the 3rd instar nymphs and adults of *A. lucorum*.【Results】The LC_{50} values of neonicotinoid insecticides to the adults and nymphs of *A. lucorum* ranged from 337.97 to 496.03 μg/mL in the glass-vial toxicity test, which were significantly higher than those of other classes of insecticides (0.28 – 207.26 μg/mL). However, the LC_{50} values of the neonicotinoids to both adults and nymphs ranged from 0.01 – 1.08 μg/g in the artificial diet bioassay, which were equal to or lower than those of other classes of insecticides.【Conclusion】The results indicated that the ingestion toxicity of neonicotinoids is stronger than the contact toxicity to *A. lucorum* and these insecticides should be recommended to fight against this pest. The artificial diet test can be used as an alternative resistance monitoring method for neonicotinoids in the future.

Key words: *Apolygus lucorum*; neonicotinoids; toxicity; glass-vial bioassay; artificial diet bioassay

1 INTRODUCTION

Transgenic Bt cotton has been widely planted since commercialization in 1997 to control the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), a target pest in northern China (Wu *et al.*, 2008). Initially, about 10 000 ha were planted to Bt cotton and its planting had expanded to 3.7 million ha by 2015, which accounted for 94% of the cotton cultivated in this region (James, 2016). From 1997 to 2016, the frequency of application and amount of chemical pesticides applied against cotton bollworm has been significantly reduced and the yield of cotton, income of smallholder farmers, as well as the health of the environment have been improved (Pray *et al.*, 2002; Huang *et al.*, 2010; James, 2016). However, with the widespread planting of Bt cotton and the resulting decreased insecticide use in cotton fields, the formerly secondary insect pests, mirid bugs (Hemiptera: Miridae), such as *Apolygus lucorum* (Meyer-Dür), have become key pests, both in cotton and also numerous other adjacent crops in northern China (Lu *et al.*, 2010; Li *et al.*, 2011).

A. lucorum is a typical sucking pest. Its adults and nymphs damage cotton by feeding on terminals and squares, and cause stunting of plants, as well as the abscission of blooms and bolls, resulting in considerable losses in both yield and quality (Wu *et al.*, 2002; Wu and Guo, 2005; Lu *et al.*, 2007). Currently, insecticide application is the exclusive method used to control *A. lucorum* in cotton in China, since few effective alternative control measures are available, with Bt cotton crops

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commonly requiring approximate 20 insecticide applications per cropping season (Lu *et al.*, 2007). Effective insecticides with higher contact toxicity to control plant bugs have been screened in recent years in China (Li *et al.*, 2008; Zhang *et al.*, 2009; Li *et al.*, 2009; Men *et al.*, 2011). At present, organophosphates and pyrethroids are the most widely applied foliar insecticides to manage *A. lucorum* in Northern China, since they exhibit high contact toxicity. The organophosphates malathion, chlorpyrifos, acephate and phoxim, pyrethroids bifenthrin, and organochlorine insecticide endosulfan are among those recommended for controlling *A. lucorum* in cotton fields (Zhang *et al.*, 2009; Men *et al.*, 2011). However, organophosphates have been extensively restricted in their use pattern for posing a hazard to applicators. Pyrethroids are also effective. However, a closely related species, the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), has demonstrated the ability to develop resistance to this class of insecticide (Snodgrass and Scott, 2000). Therefore, other classes of chemicals are needed for rotation to improve resistance management. Neonicotinoids are the relatively new class of insecticides and widely applied to control common insect pests, and they have higher safety to mammals than the organophosphates (Tomizawa and Casida 2005; Preetha *et al.*, 2010). Usually applied as seed treatment or foliar spray, neonicotinoid insecticides are taken up by plants and distributed systemically (Sur and Stork, 2003) to target pest herbivores, such as aphids and whiteflies (Elbert *et al.*, 2008), which consume sap and plant tissues. The neonicotinoid insecticides thiamethoxam, acetamiprid, and imidacloprid have relatively low contact toxicity against *A. lucorum* as demonstrated using glass-vial and topical application bioassays (Li *et al.*, 2008; Zhang *et al.*, 2009; Men *et al.*, 2011). Thus, neonicotinoid insecticides have not been recommended to control this pest in the cotton field in China in the past years. In contrast, acetamiprid, imidacloprid and thiamethoxam have been shown to be active against tarnished plant bugs in USA field tests. Among these, thiamethoxam was the most effective (Layton *et al.*, 2005). Furthermore, granular treatments of both clothianidin and nitenpyram at sowing have recently been shown to prevent *A. lucorum* infestations during the seedling to blooming stages of Bt cotton in China using field plot experiments (Zhang *et al.*, 2015a).

An appropriate method to evaluate the neonicotinoid insecticides with ingestion toxicity accurately is needed, with which a quantitative analysis of insecticide toxicity can be provided correctly and objectively and the information on resistance can be obtained. For example, applying a known dose of insecticide mixed into a honey solution can be used as a monitoring method to compare the responses of insect pests to neonicotinoids over time (Snodgrass *et al.*, 2008). Diet-incorporated assays have also been used for selecting the activity of orally ingested insecticides against *L. lineolaris* (Allen *et al.*, 2012; Parys *et al.*, 2016). Based on a successful artificial diet developed for rearing *A. lucorum* (Feng *et al.*, 2012), it is possible to select and evaluate the ingestion toxicity of insecticides with artificial diet bioassay. In this study, 16 insecticides with different modes of action were selected to investigate their contact toxicities and ingestion toxicities to nymphs and adults of *A. lucorum* by glass-vial and artificial-diet bioassay methods. The main aims of this toxicology study are to get a more comprehensive understanding on the toxic effects of insecticides, especially neonicotinoid insecticides on *A. lucorum*, and to provide informative data to promote the implementation of Integrated Pest Management in Bt cotton fields, and to develop a new method for monitoring resistance.

2 MATERIALS AND METHODS

2.1 Test insect

A laboratory strain of *A. lucorum* was established by collecting nymphs and adults with a sweep net from weeds and cotton fields at Huaiyang (114.520°E, 33.440°N), Henan province of China in 2007. The population was maintained on a diet of fresh green bean under the conditions of 26 ± 1℃, 60% – 70% RH and a photoperiod of 16L∶8D, as described in Lu *et al.* (2008). A total of 150 nymphs and adults were reared on fresh green beans placed in a plastic rearing box (20 cm × 12 cm × 7 cm), with a nylon mesh top tied by rubber band to prevent escape. Fresh green beans were soaked in a 5% sodium hypochlorite solution for 10 min to remove insecticide residue, then washed using clean water and dried before placing into the box. The green beans were replaced every 2 d to ensure freshness. Rumpled wet filter paper with four layers was put into the box as the oviposition vector (Chen *et al.*, 2012). The wet filer paper with *A. lucorum* eggs was then placed into a new rearing box to hatch under the same conditions. A cotton wool absorbing 5% honey solution was placed on the nylon mesh to provide adults nutrition.

2.2 Insecticides

A total of 16 insecticides from 5 different insecticide classes tested in this study are listed in Table 1. These insecticides are widely used in agriculture in China. In order to test the effects of bioactive material of the insecticides on the mortality, technical grade insecticides instead of commercial formulations were used.

Table 1 Name, stock, and manufacturer of insecticides used in this study

Insecticide	Technical grade (%)	Manufacturer
Organophosphate insecticides		
Chlorpyrifos	94	Nanjing Red Sun Chemical Co., Ltd.
Phoxim	90	Hubei Xianlong Chemical Co., Ltd.
Malathion	90	Jiangsu Haoshoucheng Weien Chemical Co., Ltd.
Acephate	97	Jiangsu Lanfeng Chemical Co., Ltd.
Dimethoate	98	Hunan Haili Changde Chemical Co., Ltd.
Pyrethroid insecticides		
Cyhalothrin	95	Jiangsu Yangnong Chemical Co., Ltd.
Bifenthrin	96	Jiangsu Huangma Chemical Co., Ltd.
Beta-Cyhalothrin	99	Shandong Dachen Pesticide Co., Ltd.
Deltamethrin	98.5	Jiangsu Yangnong Chemical Co., Ltd.
Neonicotinoid insecticides		
Imidacloprid	95	Nanjing Red Sun Chemical Co., Ltd.
Acetamiprid	97	Jiangsu Weier Chemical Co., Ltd.
Thiamethoxam	98	Shijiazhuang Ruikai Chemical Co., Ltd.
Nitenpyram	95	Jiangsu Nantong Agro-chemical Co., Ltd.
Carbamate insecticides		
Carbosulfan	90	Jiangsu Changlong Chemical Co., Ltd.
Methomyl	98	Jiangsu Changlong Chemical Co., Ltd.
Organochlorine insecticide		
Endosulfan	94	Jiangsu Kuaida Agro-chemical Co., Ltd.

2.3 Bioassay methods for toxicity test

2.3.1 Glass-vial test: Both nymphs and adults of *A. lucorum* were tested for sensitivity to insecticides by a glass-vial bioassay developed by Snodgrass (1996). In the glass-vial bioassay, technical-grade insecticides were applied to a 20-mL glass vial by pipetting 0.5 mL of the insecticide diluted in acetone into the vial. The vial was then rolled on a hotdog cooker to evaporate the acetone, coating the insecticide residue on the inner surface. Before adding the nymphs or adults to a vial for testing, a piece of 3 mm thick green bean that had been treated as described above was placed into the vial as food. Two 3rd instar nymphs or adults of 1 – 10 day-old were placed into each treated vial. After the plant bugs were placed in the vial, the vial was capped with a cotton ball to prevent escape. During a test, the vials were held in the laboratory in an upright position under the conditions of 26 ± 1℃ and 60% – 70% RH and a photoperiod of 16L:8D.

A concentration range for each insecticide (causing 0 – 100% mortality) was obtained by conducting a preliminary test. At least five doses (as many as nine) and a control were tested in three replications. Each vial contained two adults or nymphs with five vials of each replication. Control vials were treated only with 0.5 mL acetone, and control mortality was rare and never >3.3%. Mortality was determined 24 h later, and nymphs or adults were considered dead if they were unable to right themselves or walk, or there was no movement when prodded.

2.3.2 Artificial diet test: A modified Ni artificial diet called T7 was used for these experiments and the detailed information about the components and preparation process followed the process detailed in Feng *et al.* (2012). For each tested concentration, the desired amount of insecticide was dissolved in 1 mL acetone and incorporated in a 100 g artificial diet. The diets were sealed with a vacuum sealer in triangle plastic bags (9 cm × 14 cm × 17 cm).

The feeding process was the same as described in Feng *et al.* (2012). Diet packs were made using about 1 g (about 3 drops) of each diet onto the center of a piece of square parafilm (~1 cm^2), which was stretched to about 10 times its original size, and sealed by twisting the four corners with hands. The diet packs were then placed into a clean 20-mL glass vial. Two adults or nymphs were then transferred into the tube with a fine brush pen. After plant bugs were placed in a vial, a cotton ball was placed in the vial opening to confine the bugs. During a test, the vials were held in the laboratory in an upright position under the conditions of 26 ± 1℃, and 60% – 70% RH and a photoperiod of 16L∶8D. Mortality was determined 48 h later, and nymphs and adults were considered dead if they were unable to right themselves or walk, or there was no movement when prodded.

A range of insecticide concentrations in artificial diet were used in pre-trials to determine a concentration range that resulted in 0 – 100% mortality. At least five doses (as many as nine) were tested, and each test was replicated three times. Each replication had five vials, each containing two adults or nymphs. Control diets were treated only with 1 mL acetone in 100 g diet and control mortality was rare and never >3.3%.

2.4 Statistical analysis

Data were corrected for control mortality using Abbott's formula (Abbott, 1925) before analysis. Probit analyses (Finney, 1971) of the mortality data were performed using POLO-PC (LeOra Software, 2003) to estimate LC_{50} and LC_{90} values and the slopes of the dose-response curves. Significant level of mean separation ($P < 0.05$) was based on non-overlap between the 95% confidence limits of two LC_{50} values.

3 RESULTS

3.1 Contact toxicities of 16 insecticides to nymphs and adults of *A. lucorum* in the glass-vial test

The toxicities of 16 insecticides to nymphs and adults of *A. lucorum* assessed by the glass-vial contact test are presented in Tables 2 and 3. Different insecticides varied widely in their contact toxicities, and different insecticides within the same chemical classes had different toxicities to both nymphs and adults of *A. lucorum*. Among the tested insecticide classes, neonicotinoids were the least toxic. The LC_{50} values of neonicotinoid insecticides to the nymphs and adults of *A. lucorum* ranged from 337.97 to 496.03 μg/mL in the glass-vial test, which were significantly higher than those of other classes of insecticides (0.28 – 207.26 μg/mL). The descending order of toxicity of 16 insecticides to *A. lucorum* nymphs was ranked as follows: bifenthrin > methomyl, chlorpyrifos, malathion ⩾ endosulfan, phoxim, carbosulfan > acephate, dimethoate, cyhalothrin, deltamethrin ⩾ beta-cyhalothrin ⩾ acetamiprid, imidacloprid, nitenpyram, and thiamethoxam. The toxicity of bifenthrin was 1 771-fold higher than that of thiamethoxam at a 24-h interval. The descending order of toxicity of 16 insecticides to *A. lucorum* adults was ranked as follows: bifenthrin > methomyl, chlorpyrifos > malathion, phoxim, endosulfan > carbosulfan, acephate, dimethoate > cyhalothrin, deltamethrin, beta-cyhalothrin > thiamethoxam, acetamiprid, nitenpyram, and imidacloprid. The toxicity of bifenthrin was 674-fold higher than that of imidacloprid at a 24-h interval.

Table 2 LC_{50} and LC_{90} values of 16 insecticides to the 3rd instar nymphs of *Apolygus lucorum* in the glass-vial test

Insecticides	n	Slope (*SE*)	LC_{50} (95% CI) (μg/mL)	LC_{90} (95% CI) (μg/mL)	χ^2	*df*
Organophosphate insecticides						
Chlorpyrifos	210	3.58 (0.72)	2.46 (1.83 – 3.08)	5.62 (4.29 – 9.32)	4.79	5
Phoxim	210	2.67 (0.35)	6.86 (5.48 – 8.79)	20.71 (14.93 – 34.22)	4.56	5
Malathion	180	2.80 (0.39)	4.32 (3.07 – 6.78)	12.39 (7.63 – 34.63)	3.34	4
Acephate	150	3.47 (1.02)	26.53 (18.65 – 36.15)	62.11 (42.95 – 199.14)	6.36	3
Dimethoate	150	2.46 (0.33)	40.33 (30.48 – 53.23)	134.11 (92.92 – 248.52)	4.23	3
Pyrethroid insecticides						
Cyhalothrin	150	1.65 (0.54)	50.26 (8.31 – 95.65)	167.83 (140.71 – 200.09)	5.36	3
Bifenthrin	120	2.56 (0.52)	0.28 (0.18 – 0.37)	0.89 (0.66 – 1.50)	3.23	2
Beta-Cyhalothrin	210	2.06 (0.69)	207.26 (69.59 – 291.61)	766.42 (485.89 – 2 403.85)	5.68	5
Deltamethrin	180	2.12 (0.31)	118.46 (92.75 – 160.67)	475.79 (305.74 – 1012.89)	3.84	4
Neonicotinoid insecticides						
Imidacloprid	180	1.34 (0.24)	442.45 (277.35 – 651.05)	4 028.75 (2 197.61 – 12 855.07)	5.48	4
Acetamiprid	180	2.09 (0.31)	337.97 (258.03 – 437.61)	1 378.39 (945.11 – 2 576.68)	4.78	3
Thiamethoxam	180	1.70 (0.27)	496.03 (336.39 – 685.24)	2 815.82 (1 795.34 – 6 079.89)	3.54	4
Nitenpyram	180	1.36 (0.23)	487.96 (250.78 – 589.06)	3 376.70 (1 812.91 – 10 255.19)	2.64	4
Carbamate insecticides						
Carbosulfan	180	2.26 (0.37)	7.19 (4.57 – 9.91)	26.52 (18.92 – 44.16)	2.99	4
Methomyl	150	5.51 (0.90)	2.05 (1.67 – 2.48)	3.49 (2.82 – 5.27)	6.88	3
Organochlorine insecticides						
Endosulfan	210	1.98 (0.23)	5.45 (3.38 – 10.14)	24.23 (12.34 – 100.21)	5.36	5

Table 3 LC_{50} and LC_{90} values of 16 insecticides to *Apolygus lucorum* adults in the glass-vial test

Insecticides	n	Slope (*SE*)	LC_{50} (95% CI) (μg/mL)	LC_{90} (95% CI) (μg/mL)	χ^2	*df*
Organophosphate insecticides						
Chlorpyrifos	210	2.76 (0.37)	1.80 (1.41 – 2.26)	5.25 (3.96 – 8.04)	3.91	5
Phoxim	210	1.63 (0.40)	4.66 (1.01 – 9.02)	28.58 (13.57 – 472.75)	3.84	5
Malathion	210	4.21 (0.73)	3.49 (2.61 – 4.57)	7.03 (5.22 – 12.96)	6.25	5
Acephate	180	2.15 (0.30)	20.06 (12.96 – 28.35)	79.39 (52.94 – 155.61)	2.98	4
Dimethoate	150	3.97 (0.73)	27.60 (21.32 – 33.49)	58.01 (46.49 – 84.55)	3.21	3
Pyrethroid insecticides						
Cyhalothrin	120	4.10 (0.88)	48.45 (35.30 – 59.56)	99.49 (79.22 – 152.39)	5.52	2
Bifenthrin	210	5.92 (1.60)	0.70 (0.51 – 0.83)	1.15 (0.95 – 1.78)	5.71	5
Beta-Cyhalothrin	210	2.42 (0.39)	81.70 (62.07 – 104.41)	277.45 (197.74 – 494.76)	5.37	5
Deltamethrin	180	1.97 (0.37)	63.33 (38.92 – 89.34)	282.81 (182.18 – 675.12)	7.82	4
Neonicotinoid insecticides						
Imidacloprid	120	1.15 (0.33)	472.6 (471.90 – 284.86)	6 109.15 (1 749.19 – 418 006.15)	4.32	2
Acetamiprid	150	1.96 (0.33)	425.66 (305.27 – 579.13)	1 915.15 (1 237.45 – 4 160.12)	5.41	3
Thiamethoxam	150	0.99 (0.25)	370.28 (208.79 – 1108.95)	7 413.35 (1 934.30 – 356 118.45)	3.27	3
Nitenpyram	150	2.22 (0.38)	428.21 (312.05 – 634.74)	3 456.23 (1 442.28 – 4 350.12)	1.24	3
Carbamate insecticides						
Carbosulfan	270	3.66 (0.67)	18.77 (14.47 – 23.06)	42.07 (32.89 – 64.63)	7.35	7
Methomyl	210	5.27 (1.24)	1.45 (1.01 – 1.80)	2.54 (2.01 – 4.44)	9.21	5
Organochlorine insecticides						
Endosulfan	210	4.00 (0.53)	6 (3.29 – 11.95)	12.54 (7.52 – 75.47)	6.53	5

3.2 Ingestion toxicities of 16 insecticides to nymphs and adults of *A. lucorum* in the artificial diet test

The toxicities of 16 insecticides to the 3rd instar nymphs and adults of *A. lucorum* assessed by the artificial diet test are shown in Tables 4 and 5. Unlike the results of contact toxicity, each of the insecticides evaluated in the artificial diet test showed similar toxicities to *A. lucorum*. The LC_{50} values of the neonicotinoids to both adults and nymphs ranged from 0.01 – 1.08 μg/g in the artificial diet test, which were equal to or lower than those of other classes of insecticides. The order of toxicity of these insecticides to *A. lucorum* nymphs based on the LC_{50} values was as follows: nitenpyram, methomyl, bifenthrin > acetamiprid, malathion, endosulfan, imidacloprid, chlorpyrifos, phoxim > thiamethoxam, carbosulfan, acephate, deltamethrin, dimethoate, cyhalothrin, and beta-cyhalothrin. The toxicity of nitenpyram was 287.6-fold higher than that of beta-cyhalothrin at a 48-h interval. Among the four neonicotinoids tested, nitenpyram exhibited the highest toxicity, while thiamethoxam was the least toxic. Based on the LC_{50} values, nitenpyram was 21.6 times more toxic than thiamethoxam. The order of toxicity of neonicotinoids to *A. lucorum* nymphs based on the LC_{50} values was as follows: nitenpyram > acetamiprid, imidacloprid > thiamethoxam.

Similar to the toxicity to nymphs, the order of toxicity of 16 insecticides to *A. lucorum* adults based on the LC_{50} values was as follows: imidacloprid > acetamiprid, nitenpyram, thiamethoxam, bifenthrin, malathion > chlorpyrifos, endosulfan, methomyl, phoxim, acephate, deltamethrin > carbosulfan, cyhalothrin dimethoate, beta-cyhalothrin. The toxicity of imidacloprid was 648-fold higher than that of beta-cyhalothrin at a 48-h interval. The four neonicotinoids tested exhibited equal or higher toxicity as compared with other classes of insecticides. The results suggest that neonicotinoids which was ingested are more toxic to *A. lucorum* adults in artificial diet test.

4 DISCUSSION

In the glass vial test, most of conventional insecticides, including organophosphates, pyrethroids, carbamates and organochlorines, were highly toxic to adults and nymphs of *A. lucorum*, while neonicotinoid insecticides had comparatively lower toxicity to adults and nymphs of *A. lucorum* (Tables 2 and 3). However, neonicotinoid insecticides were equally toxic to adults and nymphs of *A. lucorum*

Table 4 LC_{50} and LC_{90} values of 16 insecticides to the 3rd instar nymphs of *Apolygus lucorum* in the artificial diet test

Insecticides	n	Slope (*SE*)	LC_{50} (95% CI) (μg/mL)	LC_{90} (95% CI) (μg/mL)	χ^2	*df*
Organophosphate insecticides						
Chlorpyrifos	150	1.44 (0.49)	0.74 (0.18 - 1.52)	5.79 (2.44 - 207.24)	2.20	3
Phoxim	150	1.26 (0.43)	0.84 (0.25 - 1.97)	8.69 (3.09 - 686.00)	1.00	3
Malathion	150	2.04 (0.46)	0.31 (0.15 - 0.47)	1.31 (8.43 - 29.42)	3.93	3
Acephate	150	1.13 (0.41)	1.75 (0.46 - 4.85)	23.67 (7.13 - 9865.09)	2.77	3
Dimethoate	120	1.93 (0.53)	5.50 (2.65 - 8.76)	25.35 (143.14 - 1196.17)	2.07	2
Pyrethroid insecticides						
Cyhalothrin	150	1.34 (0.44)	5.87 (1.37 - 11.37)	53.26 (23.18 - 1 156.93)	2.75	3
Bifenthrin	180	0.99 (0.26)	0.15 (0.05 - 0.33)	2.86 (0.99 - 47.27)	2.97	4
Beta-Cyhalothrin	210	1.76 (0.46)	14.38 (5.76 - 21.58)	76.64 (48.59 - 240.38)	5.78	4
Deltamethrin	180	1.29 (0.34)	4.15 (1.25 - 7.67)	40.82 (19.75 - 254.71)	3.16	4
Neonicotinoid insecticides						
Imidacloprid	180	1.21 (0.31)	0.38 (0.11 - 0.72)	4.41 (2.03 - 30.84)	3.82	4
Acetamiprid	150	2.26 (0.55)	0.17 (0.08 - 0.26)	0.63 (0.41 - 1.43)	5.66	3
Thiamethoxam	180	5.65 (1.69)	1.08 (0.64 - 1.32)	1.82 (1.51 - 2.74)	6.44	4
Nitenpyram	180	2.09 (0.48)	0.05 (0.03 - 0.08)	0.21 (0.14 - 0.51)	4.83	4
Carbamate insecticides						
Carbosulfan	120	1.75 (0.50)	1.20 (0.78 - 3.23)	10.77 (5.98 - 56.31)	1.64	2
Methomyl	150	1.60 (0.52)	0.12 (0.03 - 0.23)	0.76 (0.40 - 9.80)	2.76	3
Organochlorine insecticides						
Endosulfan	150	1.34 (0.30)	0.37 (0.18 - 0.66)	3.33 (1.66 - 15.47)	3.57	3

Table 5 LC_{50} and LC_{90} values of 16 insecticides to *Apolygus lucorum* adults in the artificial diet test

Insecticides	n	Slope (*SE*)	LC_{50} (95% CI) (μg/mL)	LC_{90} (95% CI) (μg/mL)	χ^2	*df*
Organophosphate insecticides						
Chlorpyrifos	180	1.48(0.32)	0.17(0.07 - 0.30)	1.27(0.72 - 3.58)	4.88	4
Phoxim	150	1.50(0.48)	0.75(0.24 - 1.39)	5.33(0.30 - 1.00)	3.51	2
Malathion	150	2.06(0.51)	0.11(0.04 - 0.17)	0.45(0.30 - 1.00)	3.14	3
Acephate	180	0.77(0.24)	0.76(0.10 - 1.92)	35.57(9.36 - 3 952.38)	3.12	4
Dimethoate	120	1.64(0.50)	4.43(1.59 - 7.38)	26.90(13.95 - 233.52)	1.86	2
Pyrethroid insecticides						
Cyhalothrin	150	1.74(0.47)	3.16(0.89 - 5.47)	17.25(0.19 - 52.89)	4.22	3
Bifenthrin	180	1.02(0.26)	0.06(0.01 - 0.13)	1.11(0.47 - 8.94)	1.28	4
Beta-Cyhalothrin	150	1.47(0.44)	6.48(2.21 - 11.09)	47.99(23.73 - 449.10)	4.22	3
Deltamethrin	180	0.92(0.26)	1.10(0.12 - 2.48)	27.11(11.67 - 289.72)	4.81	4
Neonicotinoid insecticides						
Imidacloprid	180	1.23(0.29)	0.01(0.00 - 0.02)	0.33(0.15 - 1.97)	6.51	3
Acetamiprid	210	1.63(0.26)	0.03(0.02 - 0.05)	0.21(0.13 - 0.48)	9.25	4
Thiamethoxam	210	1.48(0.28)	0.05(0.03 - 0.09)	0.37(0.17 - 2.20)	3.97	4
Nitenpyram	210	1.33(0.23)	0.03(0.01 - 0.04)	0.23(0.12 - 0.86)	2.44	4
Carbamate insecticides						
Carbosulfan	120	2.22(0.71)	2.67(0.90 - 4.13)	6.77(4.26 - 21.67)	5.46	2
Methomyl	150	1.14(0.35)	0.55(0.11 - 1.07)	7.33(3.10 - 129.14)	5.56	3
Organochlorine insecticides						
Endosulfan	150	1.24(0.32)	0.28(0.08 - 0.46)	1.49(0.88 - 3.93)	7.13	3

as compared with other conventional insecticides in an artificial diet test (Tables 4 and 5). These results indicated that the toxicity estimation of an insecticide differs among methods. The glass-vial test is an initial screening technique used to assess the relative toxicity of chemicals to *A. lucorum* in that insecticides are absorbed mainly by the cuticle (Zhang *et al.*, 2009; Men *et al.*, 2011); however, it fails to represent the situations where the insecticides are absorbed mainly by sucking. This is especially pertinent for neonicotinoid insecticides, which are systemic in plants (Sur and Stork, 2003). Therefore, the artificial diet test is more adequate to evaluate the toxicity of neonicotinoids to *A. lucorum*.

The organophosphate insecticides chlorpyrifos, phoxim and malathion displayed high toxicity to adults and nymphs of *A. lucorum* using both methods, indicating that these insecticides have equal contact and stomach toxicity. Similar to those used to control *A. lucorum*, organophosphate insecticides recommended by the Cooperative Extension Service to control tarnished plant bug in USA cotton include acephate and malathion (Snodgrass *et al.*, 2009). In some areas, however, the tarnished plant bug has evolved resistance to acephate. Furthermore, the inheritance of resistance in these areas to acephate was not sex-linked and the alleles controlling the resistance were semi-dominant in nature. This makes this resistance persistent, easy to select for, and likely to spread to other cotton growing areas (Snodgrass *et al.*, 2009). Although *A. lucorum* have been shown to be sensitive to organophosphate insecticides in Shandong and Henan province based on the monitoring data available (Guo *et al.*, 2010; Zhang *et al.*, 2013; Li *et al.*, 2015; Zhang *et al.*, 2015b), monitoring the development of resistance to organophosphates in *A. lucorum* should not be ignored. Similar to organophosphates, in our study, the carbamate insecticides carbosulfan and methomyl, and the organochlorine pesticide endosulfan, were shown to have relatively high contact toxicity and ingestion toxicity to the adults and nymphs of *A. lucorum* using both glass-vial and artificial diet tests.

Pyrethroids bind to a distinct receptor site on the sodium channel and prolong the open state by inhibiting channel activation and inactivation, and these compounds have high insecticidal activities through both contact and stomach actions (Bloomquist, 1996). However, except bifenthrin, cyhalothrin, beta-cyhalothrin, and deltamethrin were relatively less toxic to *A. lucorum* as compared with organophosphates in both the artificial diet and glass-vial tests. Bifenthrin has been recommended to control *A. lucorum* in cotton fields (Guo *et al.*, 2010, Zhang *et al.*, 2013, Zhang *et al.*, 2015b), and was the most toxic pyrethroid tested. Pyrethroid resistance was found to be widespread in plant bug populations in the delta region of Arkansas, Louisiana, and Mississippi in the USA (Hollingsworth *et al.*, 1997; Snodgrass and Scott, 2000). This resistance changed throughout the growing season, with the most resistant populations found in the fall (after exposure to insecticides in cotton during the growing season) as compared with in spring (Snodgrass and Scott, 2000). Therefore, monitoring the sensitivities of plant bugs collected in the fall to pyrethroid insecticides is critical for detecting resistant populations early.

Neonicotinoids are among the most effective insecticides for the control of sucking and chewing pests. They act as competitive inhibitors on nicotinic acetylcholine receptors (nAChR) in the central nervous system (Elbert *et al.*, 2008). Imidacloprid, acetamiprid, nitenpyram, and thiamethoxam were relatively highly toxic to both adults and nymphs of *A. lucorum* in an artificial diet test among the five chemical classes tested, but they were of relatively low toxicity to *A. lucorum* in the glass-vital test, which confirms the results obtained by Zhang *et al.* (2009). The toxicity difference of these insecticides assessed by the two methods can explain why acetamiprid, imidacloprid and thiamethoxam have been shown to be active against plant bugs in field trials (Layton *et al.*, 2005; Zhang *et al.*, 2015a). It should be noted that the efficacy of neonicotinoid insecticide seed treatments to manage *A. lucorum* decreased with time in the field (Zhang *et al.*, 2015a), so during the late growing season, the use of foliar sprays of organophosphate and pyrethroid insecticides may be necessary in Bt cotton to maintain the density below the economic threshold, and to maintain a sound IPM program.

The past studies focused on using glass-vial and topical application bioassays to determine the toxicity of insecticides to plant bugs. Comparing the results obtained by the two above bioassay methods used, no remarkable differences were found among the highest toxicities of all the tested insecticides (Zhang *et al.*, 2009). Both bioassays are designed to test the contact toxicity of insecticides. The topical application is the recommended method for a standard resistance test by FAO (Tang, 1993) and the glass-vial bioassay was widely used to determine insecticide resistance levels in the tarnished plant bug populations and *A. lucorum* populations (Snodgrass, 1996; Hollingsworth *et al.*, 1997; Snodgrass and Scott, 2000; Snodgrass *et al.*, 2009; Tan *et al.*, 2012; Zhang *et al.*, 2013; Li *et al.*, 2015; Zhang *et al.*, 2015b). However, these two methods are not appropriate to evaluate the ingestion toxicity of systemic insecticides, like neonicotinoids, since they rely on contact activity. In conclusion, the artificial diet test can be used as an alternative resistance monitoring method to determine the resistance levels of insect pests to neonicotinoid insecticides in the future.

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应用瓶膜法和人工饲料混合法测定比较 16 种杀虫剂对绿盲蝽的毒力

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摘要：【目的】绿盲蝽 *Apolygus lucorum* (Meyer-Dür) 是我国 Bt 棉田内一类重要的刺吸性害虫。有机磷类和菊酯类杀虫剂因其对绿盲蝽具有较高的触杀毒力，是当前 Bt 棉田内广泛应用的杀虫剂，而新烟碱类杀虫剂因其较低的触杀毒力而没有被推荐使用。但在田间试验中，用新烟碱类杀虫剂处理棉种能够有效减少苗期至开花期绿盲蝽的危害。因此，亟待需要一种准确的毒力测定方法，以重新评价新烟碱类杀虫剂对绿盲蝽的毒力。【方法】本研究在成功研制绿盲蝽人工饲料的基础上，应用瓶膜法和人工饲料混合法两种毒力测定方法测定比较了 5 类 16 种杀虫剂对绿盲蝽 3 龄若虫和成虫的毒力。【结果】应用瓶膜法，新烟碱类杀虫剂对绿盲蝽成虫和若虫的 LC_{50} 值在 337.97～496.03 μg/mL 范围内，显著高于其他类型的杀虫剂（LC_{50} 值范围在 0.28～207.26 μg/mL）。然而，应用人工饲料混合法，新烟碱类杀虫剂对绿盲蝽成虫和若虫的 LC_{50} 值在 0.01～1.08 μg/g，与其他类杀虫剂 LC_{50} 值相等甚至低于其他类杀虫剂。【结论】结果表明新烟碱类杀虫剂对绿盲蝽的胃毒毒力显著强于它的触杀毒力，可用于绿盲蝽的田间防治。推荐人工饲料混合法作为监测绿盲蝽对新烟碱类杀虫剂抗性发展的一个替代方法。

关键词：绿盲蝽；新烟碱类杀虫剂；毒力；瓶膜法；人工饲料法

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